SMC 9/08/08 On page 189, please replace lines 8-15, with the following

- - VK-TERM-FOR

5' TGG AGA CTG GGT GAG CTC AAT GTC GGA GTG AGA ATA GAA AGG 3' **(SEQ ID NO:52)** (overlapping with VK2BACK [example 14])

and

CH1-TERM-BACK

5'AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA GTT GAG CCC AAA TCT AGC TGA TAA ACC GAT ACA ATT AAA GGC 3' **(SEQ ID NO:53)** (overlapping with HuIgG1-4 CH1-FOR)- -

SMC 9/08/08 Please replace the paragraph bridging page 191 (starting at line 28) through page 192 (line 14) with the following rewritten paragraph:

- - Aliquots of the ligation reaction were transformed into competent TG1/pUC19gIII and plated on SOB medium containing ampicillin at 100μg/ml and kanamycin at 50μg/ml. Colonies were screened for the presence of a deletion by PCR with primers gIIIFUBA and KSJ12 (CGGAATACCCAAAAGAACTGG) **(SEQ ID NO:54).-** -

On page 192, please replace lines 5-16, with the following rewritten paragraph:

- - KSJ 12 anneals to gene VI which is immediately downstream of gIII in the phage genome, so distinguishing gIII on the helper phage from that resident on the plasmid. Three clones gave **[truncated] trunctated** PCR products corresponding to deletions of ca. 200, 400 and 800bp. These clones were called M13K07 gIII Δ Nos 1,2 and 3 respectively. No clones were isolated from the earlier Bal 31 time points, suggesting that these are in some way lethal to the host cell. Several clones were isolated from later time points, but none of these gave a PCR product, indicating that the deletion reaction had gone too far.- -

On page 193, please replace lines 5-18, with the following rewritten paragraph:

- - Only a minute fraction of the gIII protein on the M13K07-rescued material is present as an intact fusion **[fig 38](Figs. 38A-38B)**. The fusion protein band is induced by IPTG, so is indisputably that synthesised by the phagemid. As expected, even when the lac promoter driving gIII

VK3F2NOT, 5'-TTC TGC GGC CGC CCG TTT CAG CTC GAG CTT GGT CCC -3' **(SEQ ID NO:50)**.

Restriction sites are underlined.

Rescue of Phage and Phagemid particles - -

SMC 9/07/08 Please replace the paragraph bridging page 160 (starting at line 26) through page 161 (line 7) with the following rewritten paragraph:

- - The phagemid vector, pHEN1 **[(fig. 26(a))]** **Figure 26(a)**, is based upon pUC119 and contains restriction sites (SfiI and NotI) for cloning the fusion proteins. Here the transcription of antibody-g3p fusions is driven from the inducible lacZ promoter and the fusion protein targetted to the periplasm by means of the pelB leader. Phagemid was rescued with VCSM13 helper phage in 2xTY medium containing no glucose or IPTG: under these conditions there is sufficient expression of antibody-g3p. Fab and scFv fragments of NQ10.12.5 cloned into pHEN1 for display were shown to bind to phOx-BSA (but not BSA) by ELISA (Table 5) using the same criterion as above.- -

SMC 9/07/08 On page 179, please replace lines 10-15 with the following rewritten paragraph:

- -The construct fdphoAla166 (derived in example 11) was converted back to the wild type residue (arginine) at position 166 by in vitro mutagenesis (Amersham International) using the printer APARG166:5' TAGCATTTGCGCGAGGTCACA 3' **(SEQ ID NO:51)**.

This construct with the wild type insert was called fdphoArg166. --

On page 183, please replace lines 1-17, with the following rewritten paragraph:

- - Phage-enzyme or free alkaline phosphatase (83ng) mixed with vector phage were passed through filters with a nominal molecular weight limit of 300,000 daltons (Ultrafree-MC filters, Millipore). Figure **[35 A]** **35A** again shows that the band of Mr, 115,000 is the major product reactive with anti-BAP antiserum. This and the other minor products reactive with anti-BAP are present in material retained by the ultrafiltration membrane. Analysis of retained and flow through fractions of phage preparations derived from KS272 demonstrates that different molecular species are being separated by the ultrafiltration membranes. Figure **[35b]** **35B** shows the protein of Mr 115,000 is retained by the filter whereas the putative degradation products of Mr 95,000 and 60,000 found in phage preparations derived from KS272 cells, are not retained.- -

different Vk genes (a to g) (Fig. 24). Most of the domains, such as VH-B and Vk-d were 'promiscuous', able to bind hapten with any of several partners.- -

SMC 6/07/08

Please replace the paragraph bridging page 148 (starting at line 10) through page 149 (line 9) with the following rewritten paragraph:

- -The sequences of the V-genes were related to those seen in the secondary response to phOx, but with differences (Fig. 24). Thus phOx hybridomas from the secondary response employ somatically mutated derivatives of three types of Vk genes - Vkoxl. 'Vkox-like' and Vk45.1 genes (C. Berek, G. M. Griffiths & C. Milstein Nature 316 412-418 (1985). These can pair with VH genes from several groups, from Vkoxl more commonly pairs with the VHoxl gene (VH group 2. R.Dildrop uupra). Vkoxl genes are always, and Vkox-like genes often, found in association with heavy chains (including VHoxl) and contain a short five residue CDR3, with the sequence motif Asp-X-Gly-X-X **(SEQ ID NO:37)** in which the central glycine is needed to create a cavity for phOx. In the random combinatorial library however, nearly all of the VH genes belonged to group 1, and most of the Vk genes were ox-like and associated with VH domains with a five residue CDR3, motif Asp/Asn-X-Gly-X-X **(SEQ ID NO:38)** (Fig. 24). Vkoxl and VHoxl were found only once (Vk-f and VH-E), and not in combination with each other. Indeed Vk-f lacks the Trp91 involved in phOx binding and was paired with a VH (VH-C) with a six residue CDR3.- -

Please replace the paragraph bridging page 151 (starting at line 9) through page 152 (line 8) with the following rewritten paragraph:

- -The promiscuity of the VH-B and Vk-d domains prompted the applicants to force further pairings, by assembling these genes with the entire repertoires if either Vk or VH genes from the same immunised mice. The resulting 'hierarchical' libraries, (VH-B x Vk-rep and VH-rep x Vk-d), each with $4x10^7$ members, were subjected to a round of selection and hapten-binding clones isolated (Table 4). As shown by ELISA, most were strong binders. By sequencing twenty-four clones from each library, the applicants identified fourteen new partners for VH-B and thirteen for Vk-d (Fig. 24). Apart from VH-B and Vk-c, none of the previous partners (or indeed other clones) from the random combinatorial library was isolated again. Again the Vk genes were mainly ox-like and the VH genes mainly group 1 (as defined in Dildrop, R. 1984 supra), but the only examples of Vkoxl (Vk-h, -p, -q and -r) have Trp91, and the VH-CDR3 motif Asp-X-Gly-X-X **(SEQ ID NO:37)** now predominates.

Replace the paragraph on page 121, lines 1-16 with the following paragraph:

- - 1. cDNA is prepared from spleen RNA from an appropriate mouse and the VH and VLK repertories individually amplified. Separately, primers reverse and complementary to VH1FOR-2 (domain 1) and VLK2BACK (domain 2) are used to amplify an existing scFv-containing DNA by PCR. (The term FOR refers to e.g. a primer for amplification of sequences on the sense strand resulting in antisense coding sequences. The term BACK refers to e.g. a primer for amplification of sequences on the antisense strand resulting in sense coding sequences). This generates a 'linker' molecule encoding the linker with the amino acid sequence (1 letter code) $(GGGGS)_3$ **(SEQ ID NO:15)** which overlaps the two primary (VH and VLK) PCR products. - -

SMC 8/07/08 Please replace the paragraph on page 126, lines 14-21, with the following rewritten paragraph:

- - The primers anneal to the 3' end. Examples of kappa light chain primers are MJK1FONX, MJK2FONX, MJK4FONX and MJK5FONX (provided under 'Primer Sequences' below) and examples of heavy chain primers are MIGG1, 2 (CTG GAC AGG GAT CCA GAG TTC CA) **(SEQ ID NO:16)** and MIGG3 (CTG GAC AGG GCT CCA TAG TTC CA) **(SEQ ID NO:17)** which anneal to CH1.- -

Please replace the paragraph beginning at page 127, line 28, with the following rewritten paragraph:

--Purify on a 2% 1mp (low melting point agarose/TAE (tris-acetate EDTA)gel and extract the DNA to 20 µl H_2O per original PCR using a **[Geneclean]** **GENECLEAN** kit (see earlier; Bio101, La Jolla CA, USA) in accordance with the manufacturers instructions.--

Please replace the paragraph bridging page 132 (starting at line 10) through page 133 (line 8) with the following:

- - Primer sequences

Primary PCR oligos (restrictions sites underlined):

VH1FOR-2 TGA GGA GAC GGT GAC CGT GGT CCC TTG GCC CC **(SEQ ID NO:18)**

On page 75, lines 8-15, please replace the paragraph with the following rewritten paragraph:

- - **[Panel A]** **Figure 38A** samples contain the equivalent of 8μl of phagemid culture supernatant per track, and 80μl of the fd supernatant (10-fold lower phage yield than the phagemid). **[Panel B]** **Figure 38B** phagemid samples are those used in **[Panel A]** **Figure 38A** at a five-fold higher sample loading (equivalent to 40μl of culture supernatant per track) to enable visualisation of the fusion band in samples rescued with parental M13K07.- -

On page 76, lines 8-9, please replace the paragraph with the following rewritten paragraph:

- - **[Figure 44 shows]** **Figures 44a-44b show** the DNA sequence of scFv B18 (anti-NP).- -

SMC 6/07/08

On page 67, line 28, replace the paragraph with the following rewritten paragraph:

[Figure 10] Figure 10a-10d shows a map of FabD1.3 in pUC19.

On page 76, lines 23-29, please replace the paragraph with the following rewritten paragraph:

- - Figure 48(i) shows a map of plasmid pJM1-FabD1.3 which is used for the expression of soluble human Fab fragments and as a template for the synthesis of linker DNA for Fab assembly. Figure 48(ii) is a schematic representation of sequences encoding a Fab construct. Fig. 48(iii) shows the sequence of DNA template for the synthesis of linker DNA for Fab assembly.- -

On page 77, lines 5-13, please replace the paragraph with the following rewritten paragraph:

- -**[Figure 50]** **Figures 50a-50b**. ELISA assay of phage antibodies using plates coated with turkey egg lysogyme. Two clones B1 and A4 are shown derived by mutagenesis and selection from pAbD1.3 (example 45). Concentration (x axis) refers to the concentration of phage for each sample relative to the concentration in culture supernatant. B1 has raised binding to turkey egg lysogyme compared to D1.3. A4 has reduced binding to hen egg lysogyme compared to D1.3.- -

On page 81, lines 5-8, please replace the paragraph with the following rewritten paragraph:

- - This example shows how a phage (eg. pAbD1.3) displaying one sort of binding molecule can be isolated from phage (e.g. pAbNQ11) displaying another sort of **[biding] binding** molecule by affinity techniques.- -